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**PHYTOCHEMICAL SCREENING AND BIOACTIVITY OF *PIPER NIGRUM* SEED
EXTRACT AGAINST *SITOPHILUS ORYZAE* (COLEOPTERA: CURCULIONIDAE)**

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ABSTRACT

The rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae), is one of the most serious insect pest of rice seeds throughout the world. Currently, use of natural compounds from plant extracts has been suggested as alternative method for insect control to conventional synthetic insecticides. Petroleum ether, ethyl acetate, acetone and ethanol extracts of *Piper nigrum* seed were screened for secondary metabolites constituents. All extracts at concentration of 1% were also evaluated for their feeding/contact, fumigant toxicity and repellent activity against adults of *S. oryzae*. The preliminary phytochemical analysis showed the presence of alkaloids and terpenoids in petroleum ether extract. Alkaloids, terpenoids, flavonoids and tannins were found in acetone and ethanol extract. Ethyl acetate extract contained terpenoids and tannins. Furthermore, petroleum ether extract showed the strongest feeding/contact toxicity against *S. oryzae* with mortality of 100% within 5 days after treatment while the other extracts had moderate feeding/contact toxicity with mortality ranging from 60.00-75.00% at 7 days. All extracts exhibited low fumigant toxicity to the insect pest with mortality of 25.00-45.00% at 7 days. However, the 4 extracts showed high repellent activity against *S. oryzae*. The highest mean repellency of 91.00% was observed in ethyl acetate extract followed by petroleum ether extract (88.00%) and acetone extract (85.00%). The lowest mean repellency of 67% was obtained in ethanol extract. These result indicated that petroleum ether extract of *P. nigrum* seed has potential for integrated pest management programs of *S. oryzae* population.

Keywords: phytochemical, bioactivity, *Piper nigrum*, *Sitophilus oryzae*, plant extract

1. INTRODUCTION

Thailand is one of the world's largest exporters of rice. In 2016, Thailand exported 9.88 million tons of rice, valued at 154,434 million baht (Office of Agricultural Economics 2016). However, one of the main problems that occur during rice storage is the attack of insect pests. The rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae) is one of the most serious insect pests of stored rice, causing quantitative and qualitative losses (Auamcharoen et al. 2012). Currently, natural



products from plants have been suggested as alternative sources for insect control because many are selective to insect pests and have no or little harmful effects on non-target organisms and the environment. Furthermore, they are easily available and less expensive than synthetic insecticides.

Piper nigrum is a member of the Piperaceae family and a well-known medicinal plant in Thailand. It possesses a wide range of biological activities against insect pests including *S. oryzae* (Vanichpakorn et al. 2017). The insecticidal and repellent activities of petroleum ether and chloroform extracts of *P. nigrum* seed against *S. oryzae* have been reported previously (Khani et al. 2011). However, there has been no report on the bioactivity of other organic solvent extracts of *P. nigrum* seed against *S. oryzae*. Thus, the objectives of this work were to evaluate the feeding/contact and fumigant toxicities and repellent activity of petroleum ether, ethyl acetate, acetone and ethanol extracts of *P. nigrum* seed against *S. oryzae*, as well as their phytochemical analysis.

2. MATERIAL AND METHODS

2.1 Rearing of *S. oryzae*

Adults of *S. oryzae* were collected from naturally infested rice seeds. They were reared on disinfested seeds in the laboratory at $26\pm1^{\circ}\text{C}$ and 75 % RH under 12: 12 (L: D). The adults were allowed for mating and oviposition for two weeks. The insect parents were separated by sieving and the rice seeds containing the eggs were kept in the same condition until adult emergence. Seven to 10-day old adults were used in all the experiments.

2.2 Preparation of plant extract

One hundred grams of powders of *P. nigrum* seed were soaked in 500 ml of petroleum ether, ethyl acetate, acetone or ethanol at room temperature ($27\pm1^{\circ}\text{C}$) for 7 days. After filtering through a Buchner funnel, the extracts were concentrated to dryness by a rotary evaporator under low pressure. The crude extracts were transferred to Petri dish and kept at 4°C in a refrigerator until use.

2.3 Feeding/contact toxicity test

The contact/feeding toxicity of 4 extracts of *P. nigrum* seed at concentration of 1% was evaluated against adults of *S. oryzae* by grain treatment test. An aliquot of 1 ml of each extract was applied to 20 g of disinfested rice seeds in 250 ml plastic containers. Controls were treated with acetone alone. After evaporation of solvent from treated seeds, ten unsexed adults of *S. oryzae* were introduced into the plastic containers. The plastic containers were covered with cotton cloth held with rubber bands. The mortality of insects was recorded daily up to 7 days. Adults were considered dead when probed with sharp objects to no response.

2.4 Fumigant toxicity

Four extracts of *P. nigrum* seed at concentration of 1% were evaluated for fumigant toxicity against adults of *S. oryzae* according to a method described by Michelraj and Sharma (2006). A 250 ml plastic jar with screw lid was used as a fumigation chamber. A filter paper disc of 5 cm diameter



was treated with 0.5 ml of each extract and allowed to air-dry for 30 min. The treated filter paper was then attached to the under surface of the lid with adhesive tape. Ten unsexed adults were transferred to a 10 ml vial and the vial was covered with fine cloth. Four vials containing the insects were placed in the fumigant chamber and considered as four replications. The lid was closed and sealed by adhesive tape to create air tight condition in the chamber. The control consisted of a similar setup but without the extract. Insect mortality was observed at 1, 2, 3 and 4 days after treatment.

2.5 Repellent test

An area preference bioassay described by Obeng-Ofori et al. (1998) was adapted to evaluate repellent activity of 4 extracts of *P. nigrum* seed at 1% against adults of *S. oryzae*. Whatman filter paper of 9 cm diameter was divided into two equal parts. The first half was treated with 0.5 ml of each extract at concentration of 1% while the other half was treated with 0.5 ml of acetone alone as control. After evaporation of solvent, a full disc was remade by attaching the treated half and the control half with clear adhesive tape. Each filter paper disc was placed in a Petri dish. Ten unsexed adults of *S. oryzae* were released at the center of filter paper disc and the Petri dish was covered. The number of insect presented on control and treated sides were recorded at 1, 2, 3, 4 and 24 hours after treatment. Repellency rate (%) was calculated by using the following formula from Abbott (1925).

$$\text{Repellency rate (\%)} = \frac{(A-B)}{A} \times 100$$

Where A was average number of insect present on untreated portion and B was average number of insect present on treated portion.

2.6 Phytochemical Screening

Phytochemical screening of 4 extracts of *P. nigrum* seeds was carried out according to standard methods described by Harborne (1973) and Trease and Evans (2002).

2.6.1 Test for saponins

About 0.5 g of each extract was shaken vigorously with 5 ml of distilled water in a test tube and warmed. The formation of stable foam was taken as an indication of the presence of saponins.

2.6.2 Test alkaloids

About 0.5 g of each extract was stirred with 3 ml of 1% aqueous hydrochloric acid and heated in a boiling water bath for 10 minutes. The mixture was filtered while hot and treated with dragendoff's reagent. Appearance of orange red precipitate indicated the presence of alkaloids.

2.6.3 Test for terpenoids

Two milliliters of chloroform was added to 0.5 g of the extract followed by addition of 3 ml of concentrated sulphuric acid. A reddish brown colouration indicated the presence of terpenoids.

2.6.4 Test for flavonoids



About 1ml of extract was added with 1ml of sulphuric acid. Orange color formation confirmed the presence of flavonoids

2.6.5 Test for anthraquinones

About 0.5 g of each extract was taken in a dry test tube and 5 ml of chloroform was added and shaken for 5 min. The extract was filtered and the filtrate was shaken with equal volume of 10% of ammonia solution. A pink violet or red colour in the ammonical layer indicated the presence of anthraquinones.

2.6.6 Test for tannins

About 0.5 g of each extract was stirred with 10 ml of distilled water and few drops of 0.1% ferric chloride were added. Formation of green precipitate was indication of presence of tannins.

2.6.7 Test for steroids

Approximately 2 ml of each extract was added with 2 ml of chloroform followed by concentrated sulphuric acid. Formation of reddish brown ring at the junction showed the presence of steroids.

2.7 Statistical analysis

All experiments were arranged in a completely randomized design with 4 replications. All data were analyzed with one-way ANOVA, followed by Duncan's multiple range tests at $P < 0.01$. All values were represented as mean \pm S.E.

3. RESULT

3.1 Feeding/contact toxicity

Significant differences ($P < 0.01$) among treatments were observed at 3, 5 and 7 days after treatment (Table 1). Petroleum ether extract showed the strongest feeding/contact toxicity against adults of *S. oryzae* with mortality of 100% within 5 days. The other extracts gave moderate feeding/contact toxicity (60.00-75.00% mortality). No mortality was observed in the control.

Table 1: Feeding/contact toxicity of 4 extracts of *P. nigrum* seed against adults of *S. oryzae*.

Extract	Cumulative mortality (mean \pm S.E., %) ^a			
	1 day	3 day	5 day	7 day
Petroleum ether	0.00 \pm 0.00	95.00 \pm 2.88 ^a	100.00 \pm 0.00 ^a	100.00 \pm 0.00 ^a
Ethyl acetate	0.00 \pm 0.00	20.00 \pm 10.80 ^b	50.00 \pm 14.72 ^b	60.00 \pm 12.24 ^b
Acetone	0.00 \pm 0.00	22.50 \pm 10.30 ^b	67.50 \pm 2.50 ^b	75.00 \pm 2.88 ^{ab}
Ethanol	0.00 \pm 0.00	12.50 \pm 4.78 ^b	45.00 \pm 6.45 ^b	60.00 \pm 12.24 ^b
Control	0.00 \pm 0.00	0.00 \pm 0.00 ^b	0.00 \pm 0.00 ^c	0.00 \pm 0.00 ^c

^a Mortality within a column followed by the same letter are not significantly different at $P < 0.01$ by DMRT



3.2 Fumigant toxicity

The fumigant toxicity of extracts of *P. nigrum* seed against adults of *S. oryzae* is presented in Table 2. There was no insect mortality in treatments within 2 d after treatment. Significant differences ($P < 0.01$) among treatments were observed at 4 days after treatment. All the four extracts exhibited low fumigant toxicity (25.00-45.00%) against the pest.

Table 2: Fumigant toxicity of 4 extracts of *P. nigrum* seeds against adults of *S. oryzae*.

Extract	Cumulative mortality (mean \pm S.E., %) ^a			
	1 day	2 day	3 day	4 day
Petroleum ether	0.00 \pm 0.000	0.00 \pm 0.00	5.00 \pm 5.00 ^a	45.00 \pm 2.90 ^a
Ethyl acetate	0.00 \pm 0.000	0.00 \pm 0.00	7.50 \pm 4.790 ^a	32.50 \pm 2.50 ^{ab}
Acetone	0.00 \pm 0.000	0.00 \pm 0.00	7.50 \pm 4.790 ^a	37.50 \pm 2.50 ^{ab}
Ethanol	0.00 \pm 0.000	0.00 \pm 0.00	2.50 \pm 2.50 ^a	25.00 \pm 2.90 ^b
Control	0.00 \pm 0.000	0.00 \pm 0.00	0.00 \pm 0.00 ^a	10.00 \pm 5.90 ^c

^a Mortality within a column followed by the same letter are not significantly different at $P < 0.01$ by DMRT

3.3 Repellent activity

Ethyl acetate extract showed the strongest repellent activity against adults of *S. oryzae* with mean repellency of 91.00% followed by petroleum ether (88.00%) and acetone extracts (85.00%), respectively. The moderate repellent activity (67.00%) was observed from ethanol extract (Table 3).

Table 3: Repellent activity of 4 extracts of *P. nigrum* seeds against adults of *S. oryzae*.

Extract	Repellency (mean \pm S.E., %) ^a					Mean repellency (%)
	1 hour	2 hour	3 hour	4 hour	24 hour	
Petroleum ether	75.00 \pm 12.60	85.00 \pm 9.57	90.00 \pm 10.00 ^a	95.00 \pm 5.00	95.00 \pm 5.00	88.00 \pm 3.947
Ethyl acetate	90.00 \pm 5.60	90.00 \pm 10.00	95.00 \pm 5.00 ^a	85.00 \pm 9.57	95.00 \pm 5.00	91.00 \pm 3.069
Acetone	75.00 \pm 15.00	85.00 \pm 9.57	90.00 \pm 5.77 ^a	90.00 \pm 20.00	85.00 \pm 9.57	85.00 \pm 4.323
Ethanol	55.00 \pm 9.57	70.00 \pm 17.32	50.00 \pm 10.00 ^b	70.00 \pm 17.32	80.00 \pm 8.16	67.00 \pm 5.671

^a Repellency within a column followed by the same letter are not significantly different at $P < 0.01$ by DMRT

3.4 Phytochemical screening

The results revealed that alkaloids and terpenoids were present in petroleum ether extract. Terpenoids and tannins were detected in ethyl acetate extract while alkaloids, terpenoids, flavonoids and tannins were found in acetone and ethanol extracts (Table 4).

Table 4: Phytochemical analysis of 4 extracts of *P. nigrum* seed.

Components	Extract			
	Petroleum ether	Ethyl acetate	Acetone	Ethanol
Saponins	-	-	-	-



Alkaloids	+	-	+	+
Terpenoids	+	+	+	+
Flavonoids	-	-	+	+
Antraquinones	-	-	-	-
Tannins	-	+	+	+
Steroids	-	-	-	-

+ = indicates presence of components, - = indicates absence of components.

4. DISCUSSION

Plant based insecticides have long been recommended as alternatives to synthetic insecticides for pest control because these chemicals pose little threat to the environment and/or to human health. Many plants contain compounds that are known to possess fumigant, repellent, anti-feedant, anti-ovipositant and insect growth regulatory activities against insect pests (Negahban and Moharramipour 2007). The present investigation showed that petroleum ether extract of *P. nigrum* seed showed the strongest feeding/contact toxicity against adult of *S. oryzae* with mortality of 100% within 5 days followed by acetone, ethyl acetate and ethanol, respectively. These findings were similar with the observation of Khani et al. (2011) who investigated insecticidal activity of different solvent extracts from *P. nigrum* seed against *S. oryzae* and found that petroleum ether extract was the most toxic with a LC₅₀ value of 1.61 µl/g at 72 hours. Hussein et al. (2017) reported insecticidal activity of petroleum ether extract of *P. nigrum* fruit at concentration of 5% against *S. oryzae* with mortality of 84.4% at 7 days after treatment. Additionally, petroleum ether extract of *P. nigrum* has been reported to be effective against other insect pests. For example, Rasheed et al. (2005) found its insecticidal activity against the fourth instar larvae of *Aedes aegypti* and *Anopheles stephensi*. Khani et al. (2012) reported a 72 h LC₅₀ values of 12.52 µL/mL for petroleum ether extract of *P. nigrum* against larvae of *Corcyra cephalonic*. The present study also indicated that petroleum ether extract exhibited strong repellent activity against adult of *S. oryzae* with mean repellency of 88.00%. This was supported by Khani et al. (2011) who reported that petroleum ether extract of *P. nigrum* seed at concentration of 5% could repel 92.0% of adults of *S. oryzae*.

The results of phytochemical screening in our study showed that alkaloids and terpenoids were present in petroleum ether extract. Terpenoids and tannins were detected in ethyl acetate extract while alkaloids, terpenoids, flavonoids and tannins were found in acetone and ethanol extracts. These results were different from the observation of Varsha and Sonail (2014) who found alkaloids, saponins, tannins and flavonoids in petroleum ether and acetone extracts of *P. nigrum* seeds while alkaloids, saponins and tannins were detected in ethanol extract. The variation of phytochemical components in the same plant species may be attributed mainly to variation in plant variety, agroclimatic and geographical conditions. The phytochemical components in plant are mainly responsible for biological activity including insecticidal and repellent activities against insects. Khani et al. (2011) identified the chemical components in petroleum ether extract of *P. nigrum* seed by GC-MS and found that piperine (74.37%) and caryophyllene (18.53%) were major components.



The alkaloid piperine has been reported to be responsible for insecticidal activity against insect pests including *Spodoptera frugiperla*, *Diatraea saccharalis* (Tavares et al. 2011), *A. arabiensis*, *A. coluzzii*, *A. funestus*, *A. gambiae*, *A. quadriannulatus* (Samuel et al. 2016). Chaubey (2011) reported strong repellent activity of α -caryophyllene against adults of *Tribolium castaneum*. Thus, the strong insecticidal and repellent activities of petroleum extract of *P. nigrum* seed against adult of *S. oryzae* may be attributed to the present of piperine and caryophyllene. However, isolation and identification of the active components responsible for the observed insecticidal or repellent activity of petroleum ether extract of *P. nigrum* seed are necessary to study. These results also revealed that the efficacy of 4 extracts of *P. nigrum* seed for *S. oryzae* control depended on the organic solvent used for extraction, chemical components of the extract, and exposure time.

5. CONCLUSIONS

The petroleum ether extract of *P. nigrum* seed demonstrated strong feeding/contact toxicity and repellent activity against adults of *S. oryzae*. Phytochemical analysis showed the presence of alkaloids and flavonoids in petroleum ether extract. Thus, this extract has high potential to provide naturally occurring agents that may utilized for *S. oryzae* control. Further work is in progress to isolate and identify the insecticidal and repellent components of petroleum ether extract.

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